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Developmental Origin of Reproductive and Metabolic Dysfunctions: Androgenic Versus Estrogenic Reprogramming

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Abstract

Polycystic ovary syndrome (PCOS) is one of the most common fertility disorders, affecting several million women worldwide. Women with PCOS manifest neuroendocrine, ovarian, and metabolic defects. A large number of animal models have evolved to understand the etiology of PCOS. These models provide support for the contributing role of excess steroids during development in programming the PCOS phenotype. However, considerable phenotypic variability is evident across animal models, depending on the quality of the steroid administered and the perinatal time of treatment relative to the developmental trajectory of the fetus/offspring. This review focuses on the reproductive and metabolic phenotypes of the various PCOS animal models that have evolved in the last decade to delineate the relative roles of androgens and estrogens in relation to the timing of exposure in programming the various dysfunctions that are part and parcel of the PCOS phenotype. Furthermore, the review addresses the contributory role of the postnatal metabolic environment in exaggerating the severity of the phenotype, the translational relevance of the various animal models to PCOS, and areas for future research.

Keywords

Infertility; PCOS; fetal programming; androgens; estrogens

More than 70 million people globally experience infertility.[1] Among couples of childbearing age seeking medical help, in ~30 to 40% of the cases, it is exclusively a problem with the woman. Infertility disorders such as premature ovarian failure leading to early estrogen deficiency may lead to adverse consequences such as osteopenia, cardiovascular risk, and cognitive deficits. Because infertility can negatively impact quality of life and psychosocial well-being, approaches to prevent/overcome infertility must be developed.

Among fertility disorders, polycystic ovary syndrome (PCOS) is one of the most common. Economic burden of PCOS exceeds several billion dollars annually in the United States. A large percentage of women with PCOS do not respond to ovulation induction protocols.[2] Even if successful ovulation is induced, conception rates are low and the percentage of pregnancies ending in spontaneous miscarriages is high.[3,4] Women with PCOS are also at

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risk for ovarian hyperstimulation and multiple gestations.[4–6] They are more likely to develop gestational diabetes and preeclampsia[6] and show psychological disturbances.[7,8] Overall, they have a lower degree of satisfaction about health and sexuality.[7,8] About 70% of these women manifest insulin resistance,[9] and insulin-lowering drugs reduce hyperandrogenism implicating a metabolic component in the etiology of PCOS.[10–12] An increased risk of cardiovascular disease, dyslipidemia, hypertension, diabetes mellitus, and endometrial cancer in PCOS[13,14] emphasizes the need not only to address the issues of infertility but also the long-term goals of preventing debilitating diseases and most importantly the transgenerational transfer of unwanted traits to the offspring. The etiology of PCOS is unknown and remains a topic of intense research.

Increasing evidence suggests that adult dysfunctions may result from abnormal programming of developing systems during intrauterine life.[15] Some believe that androgen excess early in life may lead to the manifestation of PCOS in adulthood.[16,17] In support, the PCOS phenotype is associated with conditions such as classical 21-hydroxylase deficiency in which the fetus has been exposed to high concentrations of sex steroids before birth.[18] Several animal models have evolved to determine the impact of perinatal exposure to steroids on the development of adult reproductive and metabolic pathologies.[19] Many of these animal models that manifested the PCOS phenotype involved perinatal treatment with testosterone (T). These perinatal T-treated models are often referred to as androgenized models, overlooking the ability of T to be aromatized to estrogen and then exerting its effects via estrogenic programming. Other models involve perinatal exposure to dihydrotestosterone (DHT), a nonaromatizable androgen, or estrogenic agents. This review focuses on animal models that have evolved in the last decade to (1) compare and contrast the reproductive and metabolic phenotypes of these animal models relative to women with PCOS and the nonhuman primate model for PCOS, (2) delineate the relative roles of androgens and estrogens in facilitating the various disruptions, (3) address the relative strengths and weaknesses of the different models, (4) pinpoint the translational significance of these animals to human PCOS, and (5) point to future directions to be taken.

DEVELOPMENTAL PROGRAMMING OF PCOS PHENOTYPE WITH PERINATAL T EXCESS

Studies assessing developmental effects of T focused on three species, Rhesus monkeys, sheep, and rats. Monkey and sheep studies have addressed the effects of T excess starting at two different gestational time points, early and late gestation. Rat studies have addressed exposure during prenatal and early postnatal periods (Table 1[20–71]). These studies have found that developmental exposure to T excess leads to neuroendocrine, ovarian, and metabolic deficits (Fig. 1), the details of which are discussed next.

Neuroendocrine Studies

A common consequence of prenatal T excess is the induction of leuteinizing hormone (LH) excess in early-treated monkeys,[34,35] early-treated sheep,[48–50] and prenatal-treated rats.[66,67] Detailed characterization of LH pulse dynamics performed in ovary-intact early-treated sheep found disruption of all three feedback systems, namely estradiol (E_2)-negative,[50] E_2-positive,[49,60] and progesterone (P_4)-negative feedback. [61,62] A late shorter duration of treatment (gestational day [GD] 60 to 90) induced less severe disruptions at the E_2- positive feedback level.[49] Studies in early-treated monkeys (GD: 40 to 60 to 55 to 120) found reduced LH responsiveness to E_2.[34,40] Prenatal-treated rats (GD: 16–19)[66] and early-treated sheep[49] also manifest compro- mised E_2 positive feedback responses. In-depth studies testing E_2-negative and -positive feedback responses have not been undertaken in women with PCOS. Early-treated sheep[61,62] and early- and late-treated monkeys[41] manifest reduced

sensitivity to P_4-negative feedback, a feature seen in women with PCOS.[25,26] More recent, neuroanatomical studies have found that kisspeptin/neurokinin-B/dynorphin neuronal population may be involved in altered negative feedback sensitivity.[72] At the pituitary level, as in women with PCOS,[25] pituitary sensitivity to gonadotropin-releasing hormone (GnRH) is increased in prenatal T-treated sheep[48] and monkeys[34,40] but not in rats.[66] These differences may be a function of the study design; only studies in sheep,[48] but not rats[66] and monkeys,[34] were undertaken after ablation of endogenous GnRH action.

Ovarian Studies

At the ovarian level, prenatal T excess leads to polycystic ovarian morphology with increased ovarian weight/volume in monkeys[35,38] and sheep.[56] Morphometric studies and serial ultrasonography studies undertaken in sheep provide evidence in support of increased ovarian follicular recruitment/depletion[57] and persistence.[52,53] An increase in antral follicle number following prenatal T excess was also evident in monkeys[38] and rats.[67] However, the measures in rats and monkeys[38,67] as well as in women with PCOS[23] are based on a single time point evaluation unlike serial ovarian stereology[57]/ultrasound[52] undertaken at multiple developmental time points in sheep. It should also be recognized that rodents are polyovular and hence manifest polyfollicular morphology even when untreated. Furthermore, in addressing ovarian developmental programming, it is crucial to take into account the differences in the trajectory of ovarian differentiation. Sheep and subhuman primates are precocial with follicular differentiation completed in utero. In contrast, differentiation gets completed in rodent models only postnatally (Table 2[73–78]). In-depth evaluations performed only with ovaries of sheep model of PCOS have revealed disruptions in androgen/estrogen receptor ratios,[47] growth factor expression such as activin and follistatin,[56] and insulin receptor signaling[79] such as those seen in women with PCOS.[80,81]

Hyperandrogenemia

Studies conducted thus far document that prenatal T excess induces functional hyperandrogenism in monkeys manifested as enhanced responsiveness to human chorionic gonadotropin.[33,34] Prenatal T-treated sheep also manifest functional hyperandrogenism reflected as increased ovarian[47] and hypothalamic[82] androgen receptor expression, and polyfollicular morphology.[56,57] Studies in prenatal T-treated Sprague-Dawley rats are inconsistent in that hyperandrogenism was reported in one study[67] but not the other.[66] Both studies used the same regimen of T treatment both in terms of timing and dosage.

Cyclic Function and Fertility

Oligo-anovulation is a common feature of all three species (monkeys, sheep, and rodents) treated prenatally with T[34–36,51–53,62,67] with the degree of disruption depending on the timing of treatment, with late-treated sheep and monkeys revealing lesser disruptions than the early-treated ones.[34,51] Studies in monkeys, the only model where oocyte competence has been assessed, found that prenatal T excess reduces oocyte competence.[39] Fertility tests following natural mating have been undertaken only with late-treated sheep (early-treated animals are virilized) and reveal a 60% reduction in pregnancy rates.[55] Compromised fertility/fecundity is also a feature of women with PCOS.[20,83]

Cardiometabolic Studies

Developmentally, early-treated sheep manifested intra-uterine growth restriction (IUGR) and compensatory postnatal catch-up growth.[54] An increase in postnatal growth rate was also evidenced in early-treated monkeys before menarche,[36] although they did not manifest IUGR. Metabolic perturbations programmed by prenatal T excess include insulin resistance in late-treated monkeys,[42] early- and late-treated sheep,[63,64] and post-natal-treated rats[70,71]

but not prenatal-treated rats.[68] Early-treated older monkeys have been reported to develop pancreatic β cell dysfunction.[42,43] Increased visceral fat is another feature of early-treated older monkeys[45] and prenatal-treated rats.[68] Postnatal T treatment also increases fat mass in Wistar rats,[70] although it has no effect in Sprague-Dawley rats.[71] Both prenatal- and postnatal-treated rodent models manifest increased serum triglycerides and cholesterol[68,70] suggestive of an extended critical period. Telemetry studies performed only in sheep found early treatment leads to hypertension.[65] As such, prenatal T treatment has an impact on cardiometabolic aspects with the nature of disruptions differing between the species studied and possibly stemming from differences in timing of insult relative to organ differentiation.

Overall, the prenatal T-treated models manifest reproductive and metabolic features of PCOS consistent with the National Institutes of Health (NIH) 1990[84] (chronic anovulation and clinical and/or biochemical signs of hyperandrogenism), Rotterdam European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) 2003[85] (oligo- and/or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic ovaries; two of three), Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) 2006[86] (oligo- and/or anovulation with clinical and/or biochemical signs of hyperandrogenism) criteria, and the cardiovascular disease risk AE-PCOS statement.[87] Information is incomplete in the early postnatal T-treated rodent model[70,71] to assess if they meet any of these criteria.

DEVELOPMENTAL PROGRAMMING OF PCOS PHENOTYPE WITH ANDROGEN EXCESS

The nonaromatizable androgen DHT was used as the programming agent in three prenatal models and two postnatal models (Table 3). The prenatal models include sheep (GD: 30 to 90), Sprague-Dawley rats (GD: 16 to 19), and mice (GD: 16 to 18), and the two postnatal models involve Wistar rats treated either 3 hours after birth (single dose) or 21 days after birth (duration: 90 days). Although the potential for estrogenic effect of DHT via conversion to 3β-diol and action through estrogen receptor-β exists,[95] considering that the degree of such conversion in specific tissues/species remains unknown and is expected to be minimal, for the purpose of this review, DHT effects are discussed relative to its androgenic potential.

Neuroendocrine Studies

Detailed LH dynamics have been undertaken in sheep and rats and show that prenatal DHT treatment increases LH pulse frequency and amplitude.[66,88] Single time point measures in mice also show that prenatal DHT treatment increases plasma LH levels.[89] Detailed E_2-negative feedback studies with prenatal DHT treat- ment have only been performed in sheep, and these show that E_2-negative feedback responses are reduced,[88] similar to that of prenatal T-treated sheep.[50] E_2-positive feedback is disrupted in DHT-treated rats[66] but not sheep.[88] At the pituitary level, prenatal DHT treatment, similar to findings with prenatal T, increased pituitary sensitivity to GnRH in sheep[48] but not rats[66] possibly due to the test being conducted without blocking endogenous GnRH input in rats.

Ovarian Studies

The effect of perinatal DHT treatment in the development of polycystic ovarian (PCO) morphology is species specific. Although both prenatal and postnatal DHT-treated rats display PCO morphology,[67,91] this is not the case with sheep.[57] Similar studies with prenatal DHT have not been undertaken in monkeys or mice. Ovarian morphometric and serial ultrasonography studies performed only in sheep support a transient increase in follicular recruitment[57] but not follicular persistence.[53]

Hyperandrogenism

It remains to be resolved whether hyperandrogenism is a consistent feature of prenatal DHT-treated mice. Hyperandrogenism was reported as a consequence in one study conducted at 4 to 6 months of age.[89] In the second study performed by the same group, hyperandrogenism was not evident at 5 months of age.[90] Authors attributed the lack of hyperandrogenism in 5-month-old animals in the second study to the age when hyperandrogenism was examined (although there is overlap in age between this and the first study) or differences in the sensitivity of the T assay used (different assays were used in the two studies). The effect of prenatal DHT in Sprague-Dawley rats is also controversial, with one study manifesting hyperandrogenic status[67] and another not.[66] Hyper-androgenism is not a feature of postnatal DHT-treated rats.[70,91] Prenatal DHT-treated sheep are functionally hyperandrogenic only during fetal life (manifested by increased androgen receptors in granulosa and stromal compartments) but not during adult life.[47] These findings differ from the prenatal T-treated sheep, which shows evidence of hyperandrogenism both during fetal and adult life.[47]

Cyclic Function and Fertility

Cycle disruptions are evident in all models but differ in their attributes.[53,67,89–91] The preovulatory E_2 rise and LH surge dynamics studied only in prenatal DHT-treated sheep are not disrupted.[88] Fertility tests have not been performed in any of the pre- or post-natal-treated models possibly due to their virilized phenotype.

Cardiometabolic Studies

Reduced insulin sensitivity is also a feature of prenatal DHT-treated sheep[64] and the postnatal-treated rat models[70,91] but not the prenatal DHT-treated mice,[90] which display glucose intolerance.[90] Increased visceral fat was a feature of late[91] but not early[70] postnatal DHT-treated rats or DHT-treated mice.[90] No changes in lipid profiling were evident in both postnatal-treated rat models.[70,91]

The prenatal rat models show opposing findings with one meeting NIH, Rotterdam ESHRE/ASRM and the AE-PCOS criteria (cycle anomalies, PCO morphology, and hyperandrogenism)[67] and the other showing only cycle disruptions (there is no evidence of hyperandrogenism and the ovarian phenotype has not been tested).[66] The late postnatal rodent model[91] fits only the Rotterdam ESHRE/ASRM criteria by virtue of the cycle anomalies and PCO morphology. The prenatal DHT-treated sheep model manifests only cycle disruptions[53] but not hyperandrogenism or PCO morphology[57] and therefore does not fit any of the PCOS criteria. The jury is still out on the prenatal DHT-treated mouse model in view of the discrepancy seen in the hyperandrogenic phenotype between the two studies.[89,90] If hyperandrogenism is part of the consequence, the prenatal DHT-treated mouse model would meet the NIH, Rotterdam ESHRE/ASRM, as well as the AE-PCOS criteria.

DEVELOPMENTAL PROGRAMMING OF PCOS PHENOTYPE WITH ESTROGENS

Two different paradigms have been used to address the role of prenatal E_2 programming. These include E_2 valerate (EV) treatment beginning day 14 of neonatal life[94] or administration of letrozole, a nonsteroidal aromatase inhibitor, to block conversion of androgen to estrogen (estrogen ablation approach) beginning either at postnatal day 21 for 3 months (*early*[91]) or at postnatal day 42 for 3 weeks (*late*[92,93]).

Neuroendocrine Studies

Detailed neuroendocrine investigations have not been performed with these models. LH excess (studies performed without controlling for cycle stage) is a feature of the late letrozole-treated model.[92,93] This has not been studied in the early-treated model. In contrast, the EV model manifested low LH levels.[94]

Ovarian Studies, Cyclic Function, and Fertility

All three models display PCO morphology[91,92,94,96] but disagree relative to ovarian weight (high in early letrozole,[91] normal in late letrozole,[92] and low in EV[94]).

Hyperandrogenism

Hyperandrogenism is a common feature of both letrozole models,[91–93] whereas the EV-treated[94] showed the opposite, namely hypoandrogenism.

Cyclic Function and Fertility

Cycle dysfunction is a common feature of the EV as well as the early- and late-treated letrozole models, although they differed in their attributes.[91,92,94]

Cardiometabolic Studies

Insulin sensitivity, visceral fat, and lipid profile were normal in the early letrozole-treated rats.[91] Metabolic measures have not been studied in the other two animal models.

The EV model, which manifests cycle disruption and polycystic ovaries in the face of hypoandrogenism, meets the Rotterdam ESHRE/ASRM criteria of PCOS. Both letrozole models meet the NIH, Rotterdam ESHRE/ASRM as well as AE-PCOS criteria manifesting cyclic disruptions, hyperandrogenism, and PCO morphology. It should be noted that the adult phenotype of the two letrozole-treated models are similar in spite of differences in the timing of onset and duration of treatments. In the context of reprogramming, a limitation of the letrozole-treated model is that studies were performed immediately after stopping the treatment. As such, reported disruptions may be activational and dissipate after cessation of treatment.

ANDROGENIC VERSUS ESTROGENIC PROGRAMMING

The models discussed point to some aspects of the perinatal programming of the PCOS phenotype being driven by excess androgen and others by excess estrogen in a species-specific manner. In prenatal T-treated models, there is obvious potential for both androgenic and estrogenic programming. Sheep studies show that gestational T treatment increases both T and E_2 concentrations in female fetuses,[97] providing support that the resultant PCOS phenotype is likely the culmination of androgenic as well as estrogenic programming. Elevated fetal T levels but not estrogens were characteristics of gestational T-treated monkey fetuses.[44] Similar information is lacking in the small animal models.

To discern whether each of the reproductive and metabolic disruptions previously discussed arise from androgenic or estrogenic effects, animal models that compare the quality of steroids spanning the same developmental time points provide the only valid comparisons. Four models fit this criteria: sheep treated from GD 30 to 90 with T or DHT, rats treated on GD 16 to 19 (prenatal) with T or DHT, rats treated 3 hours postnatal with T or DHT, and rats treated 21 day postnatal with DHT or letrozole (Table 4). Because the monkey model of PCOS involved only T treatment and the mouse model only DHT, such comparisons are not possible in these models.

Comparison of studies conducted with prenatal T- and DHT-treated sheep suggest that PCO morphology, follicular persistence, ovarian hyperandrogenism, oligo-anovulation, and the E_2-positive feedback disruptions seen in adults are likely programmed by estrogenic actions, whereas LH excess, enhanced follicular recruitment, reduced sensitivity to E_2-negative feedback, increased GnRH sensitivity, and reduced insulin sensitivity are programmed via androgens. Studies in prenatal T versus DHT rat models[66,67,70] are in agreement with the sheep model[48,63,64] relative to androgenic programming of LH excess and reduced insulin sensitivity. For comparison with women with PCOS and other models, discussion of the sheep model in this review has focused on the ovary-intact model. It needs to be recognized that dissection of androgenic and estrogenic programming of E_2-positive and P_4-negative feedback systems were delineated first using the ovar- iectomized E_2-replaced prenatal T-treated model. [98,99]

In contrast to findings in the sheep model,[73] PCO morphology, oligo-anovulation, and E_2 positive feedback disruptions in both prenatal- and postnatal-treated rats[66,67,91] point to programming via androgens. Paradoxically, hyperandrogenism is an inconsistent finding between the two rat studies, which used identical paradigms in the same strain of rats.[66,67] Similarly, androgenic programming achieved via DHT or ablation of estrogen with letrozole yielded inconsistent metabolic outcomes, the former being insulin resistant and having increased visceral fat but the latter not.[91] Visceral adiposity and abnormal lipid profile in postnatal T- but not DHT-treated Wistar rats[70] is supportive of estrogenic programming of these variables.

The inconsistencies seen between species are likely a function of the timing of treatment relative to timing of organ differentiation. However, inconsistencies in outcome such as seen in the DHT- and letrozole-treated models, both enforcing androgenic programming within the same strain of rats using similar exposure periods, suggest that the degree of steroid excess or imbalance in the estrogen-to-androgen ratio might be the underlying cause in the reprogramming of reproductive and metabolic dysfunction and development of the PCOS phenotype. Information on endogenous levels of various androgens and estrogens during the programming windows are required across species to sort out differences in outcomes.

METABOLIC AMPLIFICATION OF STEROIDAL PROGRAMMING

Evidence to date suggests that PCOS women have an increased propensity toward ovulatory dysfunction in the presence of increased adiposity.[31] The prenatal T-treated monkeys[19,45] and rats,[68] similar to women with PCOS,[31] manifested increased visceral adiposity. Obesity induced by overfeeding also exaggerated reproductive defects in the sheep model of PCOS culminating in anovulation,[100] suggestive of metabolic amplification of disruptions. Increasing prevalence of childhood obesity[101] might therefore provide a metabolic platform for uncovering or amplifying prenatally experienced developmental insults. Given the high prevalence of obesity and its comorbidities, diabetes, cardiovascular diseases, and metabolic syndrome, in the United States, more studies with various animal models are required to substantiate the detrimental effects of overfeeding/excess weight gain in the development of the PCOS phenotype.

ROLE OF HYPERINSULINEMIA IN THE DEVELOPMENT AND AMPLIFICATION OF THE PCOS PHENOTYPE

From a metabolic perspective, obesity and prenatal T excess both cause insulin resistance and compensatory hyperinsulinemia. A higher percentage of women with PCOS manifest insulin resistance and are at risk for developing type 2 diabetes.[9] Lifestyle changes and weight loss that improve insulin sensitivity were found to improve ovulatory function in

these women.[102] A recent Cochrane review of 31 clinical trials found that insulin sensitizers enhance ovulation rates and improve menstrual patterns with success rates differing between studies,[103] possibly due to the heterogeneity of the PCOS population being studied and the timing of initiation of treatment relative to when the pathology was established.

Studies conducted in prenatal T-treated sheep and Rhesus monkeys also point to beneficial effects of insulin sensitizer treatment.[104,105] Treatment with rosiglitazone, an insulin sensitizer, begun during postpubertal life prevented further deterioration of reproductive function in prenatal T-treated sheep (cycles monitored over a 2-year period).[104] Studies performed with an older cohort of prenatal T-treated monkeys also found that treatment with pioglitazone, another insulin sensitizer, improved cyclic function.[105] In sheep, the beneficial effects of insulin sensitizer in improving reproductive function were evident at two levels: prevention from further deterioration of the reproductive axis and a reduction in the number of abnormally long cycles.[104] In the older monkeys the beneficial effects of insulin sensitizer were evident as normalization of menstrual cycle length.[105] Similar studies have not been undertaken with rat and mouse models.

Although improvement in reproductive function is clearly evident in prenatal T-treated sheep and monkey models,[104,105] as is the case with PCOS women,[103] the success rate has not been 100%, possibly because treatment was initiated after the pathology was established. In prenatal T-treated sheep, reproductive dysfunctions are evident postpubertally,[51,52] whereas defects in insulin sensitivity are evident much before during neonatal life.[63,64] Early insulin sensitizer treatment beginning when insulin sensitivity defects are manifested may prove to be more effective in achieving better success rates.

GENETIC VERSUS ENVIRONMENTAL INTERACTION IN PROGRAMMING THE PCOS PHENOTYPE

Clarification of underlying mechanisms by which developmental reprogramming of physiological function occurs is essential for targeting new strategies toward prevention. Both genetic and environmental factors have been implicated in the etiology of the PCOS phenotype.[106] Familial clustering in first-degree relatives of PCOS subjects[107] and higher prevalence of PCOS symptoms in monozygotic compared with dizygotic twins[108] provide support for a genetic contribution. However, to date, no gene has been implicated in the development of a PCOS phenotype. But heterogeneity of phenotypic features in different PCOS families and even within the same family points to the importance of the environmental contribution. It is becoming increasingly apparent that environmental insults during development induce persistent changes in the epigenome leading to altered gene expression and increased risk of adult diseases.[109] Interestingly, an epigenetic change, manifested as nonrandom X chromosome inactivation, has been reported in women with PCOS.[110]

Although maternal and environmental factors during development have been found to induce epigenetic alterations and reprogram the developmental ontogeny of the offspring, the interplay of epigenetics with genetics is likely the key determining factor in an individual's susceptibility to pathology. The lower than 50% prevalence of inheritance in first-degree relatives does provide support for such gene by environment interactions.[107] An understanding of the epigenetic mechanisms involved in models of PCOS would likely provide novel avenues for the prevention and treatment of PCOS and help reduce transgenerational susceptibility for acquiring the disrupted phenotype.

STRENGTHS OF DIFFERENT ANIMAL MODELS

All PCOS animal models discussed offer differing strengths. The highly compressed developmental time scale of developing rats and mice allows studies of transgenerational transfer of PCOS traits within a reasonable time frame. The transgenic approaches available in murine models are beneficial in pinpointing the site-specific role of suspected mediators. For instance, the green fluorescent protein–GnRH mouse has been a valuable resource in elucidating the direct effects of androgen and estrogen at the level of the GnRH neuron.[89] The strengths of the sheep model of PCOS are that they are amenable to a wide variety of procedural manipulations including performance of detailed/repetitive hormonal profiling, noninvasive sequential monitoring of ovarian follicular dynamics via ultrasound, multiple neurotransmitter measures in the same animal (due to the large size of the brain), studies in natural settings with behavioral interactions intact, and its cost effectiveness. The subhuman primates are closer to humans from an evolutionary perspective and share similar placentation and hence would be an optimal model. However, the number of years taken to achieve reproductive maturity and the enormity of resources required restrict feasibility of studies spanning from the time of developmental insults to adult pathological outcomes in the same animal within a reasonable time frame.

While translating the findings from any of these animals to humans, it is important to interpret the findings relative to the developmental trajectory of the organ system being studied as to whether differentiation gets completed prenatally or postnatally and the similarity of regulatory mechanisms. For instance, sheep and subhuman primates complete their ovarian differentiation in utero, but it occurs ex utero in rats and mice (Table 2). Therefore, the ovarian reprogramming that occurs in utero in sheep, primates, and humans would be subject to influence from changes in both fetal and maternal milieus, which is not the case in the postnatal rodent models. Similarly, in understanding neuroendocrine disruptions, it should be recognized that progesterone blocks generation of the LH surge in sheep, monkeys, and humans, but it is a facilitator in rodents.[111–113] In addressing studies focusing on the maternal-fetal interface, it should be taken into consideration that the placentation in sheep, rats, and mice differs from humans.

CLINICAL TRANSLATION AND PUBLIC HEATH RELEVANCE

The PCOS phenotype is associated with conditions such as classical 21-hydroxylase deficiency in which the fetus has been exposed to high amounts of sex steroids before birth,[18] suggesting that androgen excess early in life may lead to manifestation of this phenotype in adulthood. Levels of T in 40% of human female fetuses are elevated to levels similar to that of male fetuses at 19 to 25 weeks of gestation.[114] Interestingly, the gestational T-treated sheep female fetuses that manifest the PCOS phenotype are exposed to T at levels found in the male fetuses.[97]

Considering the experimental constraints in humans, animal models that manifest the PCOS phenotype are valuable resources for delineating the mechanisms contributing to the reproductive/metabolic disruptions seen in women with PCOS. More importantly, these models can serve as a testing ground for developing effective early prevention/treatment strategies to prevent/overcome reproductive/metabolic dysfunctions. The findings from these animal models may also have public health implications in the context of environmental exposures to steroid mimics. Human fetuses are subjected to abnormal steroidal programming via endocrine-disrupting chemicals in the environment such as bisphenol A and phthalates with estrogenic/antiandrogenic properties[115] as well as during disease states.[116]

FUTURE DIRECTIONS

Future studies with animal models should capitalize on the identified strengths of various models to discern the early causal signals involved in the development and progression of PCOS. Studies should target time points during development that are comparable to time points of organ differentiation in humans and strive to discover the relative fetal and maternal contributions in programming the human PCOS phenotype. Because of the potential for such PCOS traits to be carried forward to subsequent generations, transgenerational studies that focus on causal mechanisms are very much needed to help segregate genetic/epigenetic interactions and differences in individual susceptibility. If prenatal steroid excess is indeed a contributing factor in the development of human PCOS syndrome, it is conceivable that differences in timing of developmental exposure to androgens/estrogens may account for the different PCOS phenotypes with subsequent lifestyle patterns playing a role in revealing or amplifying the severity of phenotype programmed early during development.

In parallel, clinical studies should target early gestational stages and gain information on developmental changes at the maternal level and when possible capitalize on amniocentesis and postmortem samples to assess fetal contribution. Term cord blood samples may not be optimal because much of the programming on the ovary and brain may have occurred early during gestation. These human studies should be expanded to analyze the relative contribution of both androgens and estrogens because T has the ability to be aromatized to estrogen and mediate estrogenic reprogramming. More importantly, studies should capitalize on the strengths of these animal models to develop prevention and treatment strategies aimed toward improving fertility and metabolic outcomes at the level of the individual.

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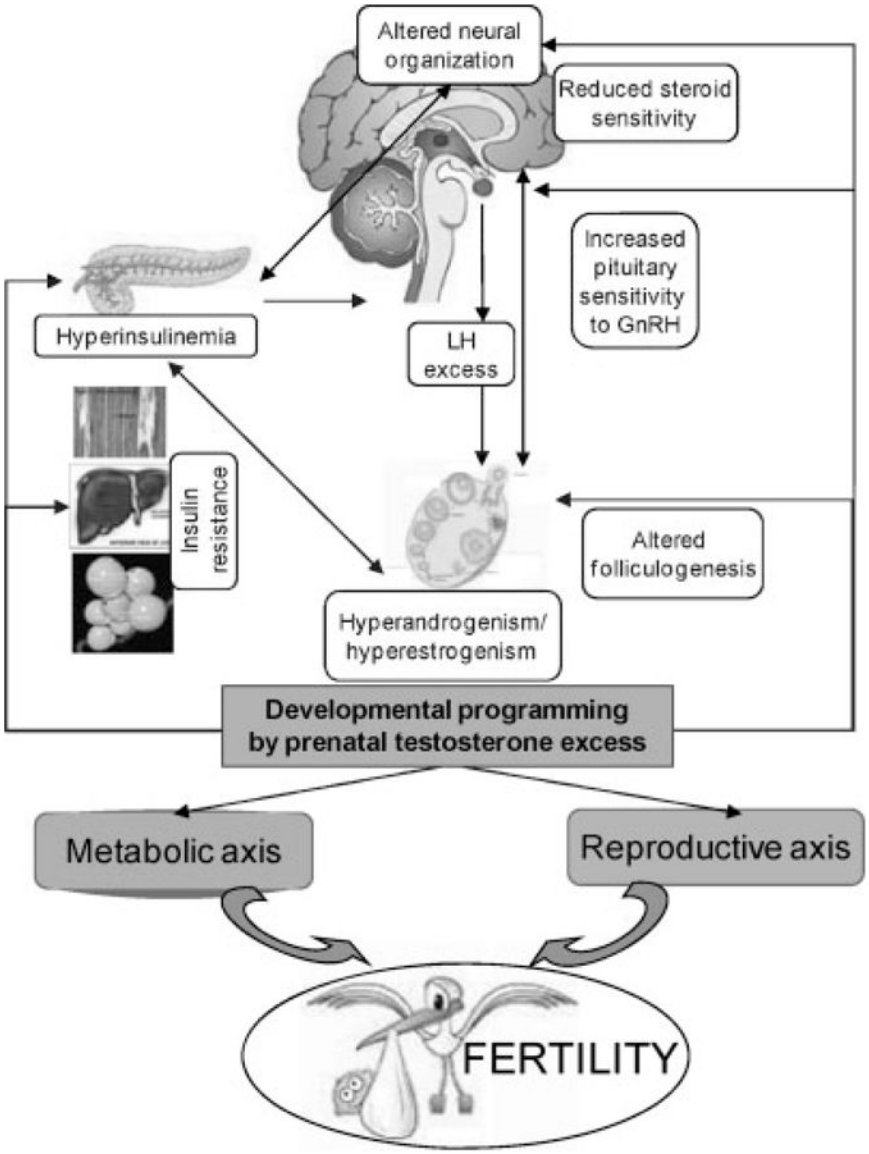

Figure 1.
Schematic showing the impact of perinatal testosterone excess on neuroendocrine, ovarian, and metabolic programming and their contribution to infertility. GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

Table 1

Comparison of Attributes of Prenatal Testosterone-Treated Monkeys, Sheep, and Rats and Postnatal Testosterone-Treated Rats with That of Women with Polycystic Ovary Syndrome

		Prenatal					Postnatal
	PCOS Women	Monkey		Sheep		Rat^{SD}	Rat$^{SD/W}$
		T^{P}		T^{P}		T^{F}	T^{P}
PCOS Phenotype		**GD (40–60) to (55–120)**	**GD (110–115) to 139**	**GD 30–90**	**GD 60–90**	**GD 16–19**	**3h PN**
Hyperandrogenism	Yes[20]	Yes^{func},[33,34]	Yes^{func},[34]	Yes^{func},[47]	–	No,[66]; yes[67]	No[70,71]
LH excess	Yes[21]	Yes[34,35]	No[34]	Yes[48–50]	No[49]	Yes[66,67]	–
Oligo-anovulation	Yes[20]	Yes[35,36]	Yes[35]	Yes[51–53]	No[51]	Yes[67]	–
Infertility	Yes[20]	Not tested, ^{V},[37]	Yes[37]	Not tested, ^{V},[54]	Yes[55]	Not tested ^{V},[66]	–
PCO morphology	Yes[22]	Yes[38]	Yes[38]	Yes[56,57]	–	Yes[67]	–
Increased ovary weight/volume	Yes[22]	Yes[35]	Yes[35]	Yes[57]	–	–	–
Follicular persistence	?	–	–	Yes[52,53]	–	–	–
Enhanced follicular recruitment	Yes^{A},[23]	–	–	Yes[57–59]	–	–	–
Increased intrafollicular androgen	Yes[24]	No[39]	No[39]	–	–	–	–
Reduced oocyte competence	Yes[22]	Yes[39]	Yes[39]	–	–	–	–
Disrupted E_2 positive feedback	?	No[40]	–	Yes[49,60]	Yes[49]	Yes[66]	–
Reduced E_2 negative feedback	?	Yes[40]	–	Yes[50]	–	–	–
Reduced P_4 negative feedback	Yes[25]	Yes[41]	Yes[41]	Yes[61,62]	–	–	–
Increased GnRH sensitivity	Yes[26]	Yes[34]	–	Yes[48]	–	No[66]	–
Reduced insulin sensitivity	Yes[9]	No[42]	Yes[42]	Yes[63,64]	Yes[64]	No[68]	Yes[70,71]
Pancreatic β-cell dysfunction	At risk[27]	Yes[42,43]	No[42]	–	–	–	–
IUGR	Yes^{B},[28]	No[44]	No[44]	Yes[54]	–	Yes^{E},[69]	–
Catch-up growth	Yes^{B},[29]	Yes^{D},[36]	–	Yes[54]	No[49]	–	–
Increased visceral fat	Yes^{C},[30]	Yes[45]	–	–	–	Yes[68]	Yes,[70] No[71]
Increased serum triglycerides	Yes^{C},[31]	–	–	–	–	Yes[68]	Yes[70]
Increased total cholesterol	Yes^{C},[31]	–	–	–	–	Yes[68]	Yes[70]

		Prenatal					Postnatal
	PCOS Women	Monkey		Sheep		Rat^{SD}	Rat$^{SD/W}$
		T^{P}		T^{P}		T^{F}	T^{P}
PCOS Phenotype		**GD (40–60) to (55–120)**	**GD (110–115) to 139**	**GD 30–90**	**GD 60–90**	**GD 16–19**	**3h PN**
Increased free fatty acids	Yes^{C},[31]	Yes[46]	–	–	–	–	No[71]
Increased atherogenic index	Yes^{C},[31]	–	–	–	–	–	Yes[70]
Hypertension	At risk[32]	–	–	Yes[65]	–	–	–

^{A}based on cortical biopsies

^{B}Spanish cohort

^{C}in obese PCOS women

^{D}prior to menarche

^{E}prenatal treatment GD16 to 20

^{F}free

^{func}functional

^{P}propionate

^{SD}Sprague-Dawley

^{V}virilized

^{W}Wistar Numbers indicate references.

PCOS, polycystic ovary syndrome; T, testosterone; GD, gestational day; LH, luteinizing hormone; PCO, polycystic ovary; E_2, estradiol; P_4, progesterone; GnRH, gonadotropin-releasing hormone; IUGR, intrauterine growth restriction.

Table 2

Schematic Showing the Time (Days) of Appearance of Different Classes of Follicles in Humans, Monkeys, Sheep, Rats, and Mice

Developmental Time Points		Human	Rhesus Monkey	Sheep	Rat	Mice
Prenatal life	Implantation	9	9	14	5.5	4
	Gonadal differentiation	42–63	40	30	12.5	6
	Start meiosis	90	60	55	17	13
	Primordial follicles	112	100	75	–	–
	Primary follicles	130	?	110	–	–
	Antral follicles	230	125	135	–	–
	Birth	270	170	147	22	20
Postnatal life	Primordial follicles	–	–	–	1–2	2–5
	Primary follicles	–	–	–	2–3	2–5
	Antral follicles	–	–	–	15	17

Data for human, monkey, sheep, and mice adapted from Padmanabhan et al.[73]

Data for the rat compiled from Kennedy et al, Pelliniemi and Fröjdman, Hemsworth and Jackson, Rajah et al, and Malamed et al.[74–78] Note in sheep, monkeys, and humans follicular differentiation is completed before birth as opposed to rats and mice, where it occurs after birth.

Table 3

Comparison of Attributes of Prenatal Dihydrotestosterone-Treated Sheep and Mice, and Postnatal Dihydrotestosterone, Letrozole, and Estradiol Valerate–Treated Rats with That of Women with Polycystic Ovary Syndrome

	Prenatal						Postnatal		
	PCOS Women	**Sheep**	**Rat^{SD}**	**Mouse**	**Rat^{W}**	**Rat^{W}**	**Rat^{W}**	**Rat^{W}**	**Rat^{W}**
		DHT^{P}	**DHT^{F}**	**DHT^{P}**	**DHT^{P}**	**DHT^{P}**	**Letrozole**	**Letrozole**	**EV**
PCOS Phenotype		**GD 30–90**	**GD 16–19**	**GD 16–18**	**3h PN**	**21d PN (90d)**	**21d PN (90d)**	**42d PN (21d)**	**14 d PN**
Hyperandrogenism	Yes[20]	No[func,47]	No,[66] Yes[67]	Yes *	No[70,89,90]	No[91]	Yes[91]	Yes[92,93]	No[94]
LH excess	Yes[21]	Yes[48]	Yes[66,67]	Yes[89]	–	–	–	Yes[92,93]	No[94]
Oligo-anovulation	Yes[20]	No[53]	Yes[67]	Yes[89,90]	–	Yes[91]	Yes[91]	Yes[92]	Yes[94]
Infertility	Yes[20]	Not tested,V,[53]	Not tested ^{V},[66]	–	–	–	–	–	–
PCO morphology	Yes[22]	No[57]	Yes[67]	–	–	Yes[91]	Yes[91]	Yes[92]	Yes[94]
Increased ovary weight/volume	Yes[22]	Yes, fetal[57]	–	–	–	No[91]	Yes[91]	No[92]	No[94]
Follicular persistence	?	No[53]	–	–	–	–	–	–	–
Enhanced follicular recruitment	Yes^{A},[23]	Yes, fetal[57]	–	–	–	–	–	–	–
Increased intrafollicular androgen	Yes[24]	–	–	–	–	–	–	–	–
Reduced oocyte competence	Yes[22]	–	–	–	–	–	–	–	–
Disrupted E_2 positive feedback	?	No[88]	Yes[66]	–	–	–	–	–	–
Reduced E_2 negative feedback	?	Yes[88]	–	–	–	–	–	–	–
Reduced P_4 negative feedback	Yes[25]	–	–	–	–	–	–	–	–
Increased GnRH sensitivity	Yes[26]	Yes[48]	No[66]	–	–	–	–	–	–
Reduced insulin sensitivity	Yes[9]	Yes[64]	–	No[90,A]	Yes[70]	Yes[91]	No[91]	–	–
Pancreatic β-cell dysfunction	At risk[27]	–	–	Yes[90]	–	–	–	–	–
IUGR	Yes^{B},[28]	–	–	–	–	–	–	–	–
Catch-up growth	Yes^{B},[29]	–	–	–	–	–	–	–	–
Increased visceral fat	Yes^{C},[30]	–	–	No[90]	No[70]	Yes[91]	No[91]	–	–
Increased serum triglycerides	Yes^{C},[31]	–	–	–	No[70]	No[91]	No[91]	–	–
Increased total cholesterol	Yes^{C},[31]	–	–	–	No[70]	No[91]	No[91]	–	–

	Prenatal				Postnatal			
	PCOS Women	**Sheep**	**Rat*SD***	**Mouse**	**Rat*W***	**Rat*W***	**Rat*W***	**Rat*W***	**Rat*W***
		DHT*P*	**DHT*F***	**DHT*P***	**DHT*P***	**DHT*P***	**Letrozole**	**Letrozole**	**EV**
PCOS Phenotype		**GD 30–90**	**GD 16–19**	**GD 16–18**	**3h PN**	**21d PN (90d)**	**21d PN (90d)**	**42d PN (21d)**	**14 d PN**
Increased free fatty acids	Yes[C,31]	–	–	–	–	No[91]	No[91]	–	–
Increased atherogenic index	Yes[C,31]	–	–	–	No[70]	–	–	–	–
Hypertension	At risk[32]	–	–	–	–	–	–	–	–

A Glucose intolerance present

F free

P propionate

SD Sprague-Dawley

V virilized

W Wistar

* See text for the dual coding of Roland et al[90] (“Developmental Programming of PCOS Phenotype with Androgen Excess,” subhead “Hyperandrogenism”).

PCOS, polycystic ovary syndrome; DHT, dihydrotestosterone; GD, gestational day; PN, postnatal; LH, luteinizing hormone; PCO, polycystic ovary; E_2, estradiol; GnRH, gonadotropin-releasing hormone; IUGR, intrauterine growth restriction.

Table 4

Androgenic versus Estrogenic Programming of the Polycystic Ovary Syndrome Phenotype*

PCOS Phenotype	Prenatal		Postnatal	
	Sheep (GD 30–90)	Rat (GD 16–19)	Rat (3-hour PN)	Rat (21 days PN)
Hyperandrogenism	Estrogenic	Inconsistent	Not disrupted	Inconsistent
LH excess	Androgenic	Androgenic	Not studied	Not studied
Oligo-anovulation	Estrogenic	Androgenic	Not studied	Androgenic
PCO morphology	Estrogenic	Androgenic	Not studied	Androgenic
Follicular persistence	Estrogenic	Not studied	Not studied	Not studied
Enhanced follicular recruitment	Androgenic	Not studied	Not studied	Not studied
Disrupted E_2 positive feedback	Estrogenic	Androgenic	Not studied	Not studied
Reduced E_2 negative feedback	Androgenic	Not studied	Not studied	Not studied
Increased GnRH sensitivity	Androgenic	Not disrupted	Not studied	Not studied
Reduced insulin sensitivity	Androgenic	Not studied	Androgenic	Inconsistent
Increased visceral fat	Not studied	Not studied	Inconsistent	Controversial
Abnormal lipid profile	Not studied	Not studied	Estrogenic	Not disrupted

* In GD 30 to 90 (prenatal) T versus DHT-treated sheep, GD 16 to 19 (prenatal) T versus DHT-treated rat, 3-hour PN T versus DHT-treated rat, and 21-day PN DHT versus letrozole-treated rat.

Assessment of androgenic or estrogenic regulation is based on outcomes described in Tables 1 and 3.PCOS, polycystic ovary syndrome; GD, gestational day; PN, postnatal; LH, luteinizing hormone; PCO, polycystic ovary; E_2, estradiol; GnRH, gonadotropin-releasing hormone; DHT, dihydrotestosterone; T, testosterone.